RECOVERY OF PALM PHYTONUTRIENTS

FIELD OF INVENTION

This invention relates to a process of recovery of phytonutrients such as carotenes, phospholipids and ubiquinones using vacuum distillation, various physical and chemical treatments and purification of the phytonutrients containing natural esterified oils and fats and has particular but not exclusive application to their recovery from palm oil.

BACKGROUND ART

Carotenoids are the natural pigments, which impart a rich orange-red colour in plants and animals. Carotenoids are found in abundance (~600 types) in nature. These include beta-carotene and alpha-carotene, which can be converted into Vitamin A (retinal) in the body. Other non-vitamin A carotenoids includes lycopene and phytoene. All these are present in crude palm oil. In fact, crude palm oil is one of the richest natural plant sources with carotenes with concentration of 500-700 ppm. Carotenoids have a number of important physiological properties. For example lycopene suppresses the growth of various cancer lines. These include the lung and liver cancer as well as colon tumours.

Ubiquinone (Coenzyme Q10) is a naturally occurring coenzyme found in palm oil. The concentration of ubiquinone in crude palm oil is determined in the range of 10-100ppm (Hazura et al. 1990). Ubiquinone is found mostly in the inner mitochondrial membrane, especially in the heart, liver, kidney and pancreas. It plays an important role in the mitochondrial electron transport chain and is also a powerful antioxidant and free radical's scavenger, and it is believed to possess membrane-stabilising properties. Since its discovery, ubiquinone has been used to aid in the treatment of many cardiovascular diseases such as congestive heart failure, cardiac arrhythmias and hypertension.

Phospholipids are essential for cell membrane repair, optimum mental function (it provides vital neurotransmitter precursor) and lipid metabolism. Phospholipids (phosphatides) are indispensable components of cell membranes and are also natural emulsifiers, helping fats dissolve in water. They support a healthy cardiovascular system and have been used as a fat emulsifier in preventing arteriosclerosis, cardiovascular disease, brain function, and proper nerve function and maintain proper electrical energy and nutrients transfer across the cell membrane.

A number of patents have been filed on the recovery of carotenes from palm oil. These include US5157132, GB2160874, US6072092 and EP0349138. The recovery processes employ esterification/ transesterification, molecular distillation, adsorbent at some stages. The current process is an advanced process integrating steps of at least one stage vacuum distillation; various physical and chemical treatment and purification to the phytonutrients concentrates. The integrated process yields higher carotenes concentration enriched with ubiquinones in indigenous diacylglycerols; and phospholipids enriched fraction.

SUMMARY OF INVENTION

This present invention relates to a process for the recovery of carotene concentrates such as carotenes, ubiquinones, and phospholipids from natural esterified oils and fats has in particular but not exclusive to crude palm oil and palm oil products.

This process involves the integration steps of (i) at least one stage vacuum distillation at temperatures ranging 80^0C-220^0C and pressure less than 40mTorr; (ii) various physical and chemical treatment including filtration, solvent partitioning, saponification re-transesterification; and (iii) purification of phytonutrients containing concentrate.

Esterification / transesterification of crude palm oil and degummed and bleached palm oil is carried out with alkyl alcohol in the presence of an alkaline catalyst under conditions sufficient to convert free fatty acids and acylglycerols into alkyl esters-rich layer is either subjected to another re-transesterification process or clean water wash for neutralisation. The esterified palm oil is subjected to one or multi-stage vacuum distillation.

After first vacuum distillation, the carotenes enriched alkyl esters (residue) is subjected to the re-transesterification process. The process is carried out with alkyl alcohol with catalyst dissolving in alcohol or clean water under sufficient conditions to convert the traces of acylglycerols into alkyl esters and glycerol. The re-transesterified alkyl esters-rich layer is then subjected to second vacuum distillation for the production of carotenes concentrate.

In some instances, the esterified and or re-esterified palm oil is subjected to one stage vacuum distillation, yielding a concentrate residue enriched in carotenes.

The carotenes enriched alkyl esters layer from the first vacuum distillation is filtered or treated with hydrocarbon solvent to remove monoacylglycerols. The filtrate is subjected to second vacuum distillation for the production of carotenes concentrate.

Mixture of carotenes concentrate could also be produced by second stage vacuum distillation alone under conditions without going through third stage vacuum distillation.

A minimum amount of palm oil ethyl esters is added to the treated carotenes enriched alkyl esters (methyl esters in this case) prior to further vacuum distillation. Carotenes concentrate produced is enriched with ubiquinones in diacylglycerols with phospholipids. Treatment of carotenes concentrate is carried out using hydrophobic and hydrophilic solvents for further purification. The concentrate could be saponified to

obtain desire concentration of carotenes fractions. Phospholipids are also recovered by membrane filtration of crude palm oil prior to conversion of oil into alkyl esters.

This present invention has many advantages. It is an integrated process where carotenes are recovered from crude palm oil, and, degummed and bleached palm oil. Carotenes recovered from this process present in diacylglycerols which is an effective carrier and dietary oil. With the improved two stage vacuum distillation, various treatments can be incorporated between the distillation stages. For instance, indigenous monoacylglycerols can be removed from the residue of first vacuum distillation after ten times of concentration and recovered as a high purity co-product. Other valuble minor components, ubiquinone and phospholipids are being concentrated in carotenes concentrate during the process.

DETAILED DESCRIPTION OF THE INVENTION

Example 1

Crude palm oil (CPO) was esterified by using sodium hydroxide as catalyst with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to molecular distillation at temperature of 110°C, wiper speed of 250rpm and pressure of 5mTorr. Residue and distillate were collected for analysis of carotenes content. The carotenes concentration was 6.5% with recovery of 80.5%. Detail results are shown in the Table 1.

Example 2

Bleached and degummed palm oil (BDPO) was esterified by using sodium hydroxide with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME was washed with hot distilled water. The neutralized BDPOME was subjected to molecular distillation at temperature of 130°C, wiper speed of 250rpm and pressure of 5mTorr. Residue and distillate were collected for analysis of carotenes content. The carotenes concentration was 12.9% with recovery of 92.5% was obtained. Detailed results are shown in the Table 2.

Example 3

Crude palm oil (CPO) was esterified by using sodium hydroxide with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to molecular distillation at temperature of 150°C, wiper speed of 300rpm and pressure of 30mTorr. All samples were analysed for carotenes content. The carotenes concentration was 5.9% with recovery of 79.9%. Detail results are shown in the Table 3.

Example 4

Bleached and degummed palm oil (BDPO) was esterified by using sodium hydroxide with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME was washed with hot distilled water. The neutralized BDPOME was subjected to molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. Residue was collected and analysed for carotenes content. The carotenes concentration was 8.5% with recovery of 91.7%. Detailed results are shown in the Table 4.

Example 5

Bleached and degummed palm oil (BDPO) was esterified by catalytic reaction with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDOME was washed with hot distilled water. The neutralised BDOME was subjected to 1st molecular distillation at temperature of 110°C, wiper speed of 250rpm and pressure of 3mTorr. Residue was subjected to 2nd molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 3mTorr. All samples were analysed for carotenes content. The carotenes concentration was 8.6% with recovery of 86%. Detail results are shown in the Table 5.

Example 6

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 250rpm and pressure of 20mTorr. Residue was re-transesterified to obtain higher degree of methyl esters conversion. The re-transesterification was carried out using sodium methylate as the catalyst. Treated sample was subjected to 2nd molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 3mTorr.The samples were analysed for carotenes and ubiquinone

content. The carotenes concentration was 14.4% with recovery of 92.7% and ubiquinone concentration was 0.3% with recovery of 94.7%. Detail results are shown in the Table 6.

Example 7

Bleached and degummed palm oil (BDPO) was esterified by catalytic reaction with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME was washed with hot distilled water. The neutralised BDPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was treated with hexane (1:1, v/v) and chilled to 0°C for two hours. The mixture was filtered and pumped dried. Treated residue was subjected to 2nd molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 12.2% with recovery of 87.9%. Detailed results are shown in the Table 7.

Example 8

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was treated with hexane (1:1, v/v) and chilled to 0°C for two hours. The mixture was filtered and washed with MeOH/$H_2$0 (5:2.5:0.5,v/v/v) for two times followed by vacuum pumped dried. Treated sample was subjected to 2nd molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 18.1% with recovery of 87.9%. Detailed results are shown in the Table 8.

Example 9

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was treated with iso-octane (1:1, v/v) and chilled to 0^0C for two hours. The mixture was filtered and pumped dry. Treated sample was subjected to 2^{nd} molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 11.0% with recovery of 88.3%. Detail results are shown in the Table 9.

Example 10

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. The residue was then subjected to re-esterification process, 50g of the concentrate was re-transesterified with 1 % alkaline catalyst (NaOH) dissolved in 20ml methanol. The mixture was refluxed at 60 - 65°C for 100 minutes. The sample of the re-esterification process was analysed for total carotenes, esters, acylglycerols and other minor components. The results of the analysis were shown in Table 10.

Example 11

The CPOME produced subjected to similar process to that of Example 10. The product produced was then subjected to re-esterification process, 50g of the concentrate was re-transesterified with 1% sodium hydroxide dissolved in 5ml distilled water. The mixture was refluxed at 60 - 65°C for 30 minutes. The sample of the re-esterification process was analysed for total carotenes, esters, acylglycerols and other minor components. The results of the analysis were shown in Table 11.

Example 12

Residue from fast speed molecular distillation of CPOME (Example 8) was added with 10% (v/v) CPO ethyl esters. The mixture was subjected to 2nd molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 1mTorr. The mass flow rate of the mixture in the distillation processes has increased 3 times of the normal flow rate without addition of ethyl esters. All samples were analysed for carotenes content. The carotenes concentration was 12.8% with recovery of 87.4%. Detailed results are shown in Table 12.

Example 13

5.0g of carotenes concentrate was subjected to unsaponification with 7.0ml of 10% potassium hydroxide in 30.0ml of ethanol. The mixture was refluxed for ½ hour. The reacted mixture was transferred to a separating funnel and the unsaponifiable matters were extracted with 50 ml of hexane: distilled water (90:10, v/v) for 3 times. The extracts were neutralised with copious of 10% ethanol in distilled water. The neutralised extract was then vacuum pumped dry and analysed. The results of the analysis are shown in Table 13.

Example 14

Carotenes concentrate (from Example 8) was used as crude material in the treatment. 0.1g of carotenes concentrate was added to 1 ml of Hexane and 3 ml of Methanol. The mixture was chilled to -10°C for 1 hour. The top and bottom layers were separated and vacuum pumped dried. Samples were analysed for total carotenes content. The carotenes concentration was 30.1% with recovery of 69%. Detail results are shown in the Table 14.

Example 15

Carotenes concentrate (from Example 8) was used as crude material in the treatment, 0.16g of carotenes concentrate was added to 5 ml of Hexane and 10ml of Methanol. The mixture was chilled to -10°C for 1 hour. The top and bottom layers were separated and vacuum pumped dry. Samples were analysed for total carotenes content. The carotenes concentration was 24.3% with recovery of 84.7%. Detail results are shown in the Table 15.

Example 16

Carotenes concentrate produced from examples 1, 3 and 4 were analysed for total phospholipids content. The results are shown in Table 16 with the concentration ranging from 0.60% to about 4.0%.

Example 17

2 litres of CPO was filtered with a membrane filter with a 0.05μm pore size. This process was carried out to reduce impurities in the CPO. These include phospholipids, iron and copper. The CPO was subjected to the membrane system with the temperature of 60°C, pressure of 2bar with 300rpm. The filtrate was analysed for total phospholipids. It was found that the total phospholipids could be reduced to 46.40ppm from 171.17ppm found in CPO.

Example 18

500g of neutralised palm oil (NPO) was esterified by sodium methylate with methanol to produce NPO methyl esters (ME). Glycerol was drained and the NPOME was divided into two portions for different neutralisation approaches. To the first part of NPOME, 10% of distilled water was used for each washing step until neutralised NPOME was obtained. To the second part of NPOME, hydrochloric acid was added into distilled water until pH 4-5. 10% of the acidified distilled water was then used for each washing step until NPOME was neutralised. The result shows that the acidified distilled water is

better than normal distilled water for neutralization of NPOME produced by reducing the total amount of distilled water used by 40%. All minor components such as carotenes, vitamin E, phytosterols and squalene were preserved well in acidified water washing. The results are shown in Table 18.

Table 1 (Single Stage Distillation – No treatment)

Condition: 110^{0}C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	65232.6	198.6	80.5

Table 2 (Single Stage Distillation – No treatment)

Condition: 130^{0}C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	129159.0	228.2	92.5

Table 3 (Single Stage Distillation – No treatment)

1st Distillation: 150°C, 300rpm, 0.93ml/min, 30mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	698.0	603.1	100.0
Carotenes Concentrate	58695.0	481.8	79.9

Table 4 (Single Stage Distillation – No treatment)

1st Distillation: 150°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	84819.0	226.2	91.7

Table 5 (Two Stage Distillation – No treatment)

1st Distillation: 110^{0}C, 250rpm, 0.9ml/min, 3mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Estesr	612.0	3172.6	100.0
Carotenes enriched alkyl esters	47174.0	2959.1	93.3

2nd Distillation: 150^{0}C, 250rpm, 0.93ml/min, 3mTorr

	Carotene		
	ppm	mg	%Recovery
Carotenes enriched alkyl esters	47174.0	2794.4	100.0
Carotenes Concentrate	86625.0	2402.5	86.0

Table 6 (Two Stage Distillation – Re-transesterification of concentrate after 1st Distillation)

1st Distillation: 90^{0}C, 200rpm, 2.2ml/min, 20mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	682.0	4124.7	100.0
Carotenes enriched alkyl esters	6796.0	4022.1	97.5

2nd Distillation: 150^{0}C, 250rpm, 0.93ml/min, 3mTorr

	Carotene			Ubiquinone		
	ppm	mg	%Recovery	ppm	mg	%Recovery
				140	60.5	100.0
Feed: Treated carotenes enriched alkyl esters	6790.0	2933.3	100.0			
Carotenes Concentrate	143123.0	2720.5	92.7	3014.0	57.3	94.7

Table 7 (Two Stage Distillation – Treatment with Hexane)

1st Distillation: 90^{0}C, 200rpm, 2.2ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	2466.7	100.0
Carotenes enriched alkyl esters	3949.4	2149.7	87.1

2nd Distillation: 150^{0}C, 250rpm, 0.93ml/min, 7mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Treated carotenes enriched alkyl esters	3949.4	1023.7	100.0
Carotenes Concentrate	121825.0	900.2	87.9

Table 8 (Two Stage Distillation – Treatment with Hexane and MeOH/H_20 Washing)

1st Distillation: 90°C, 200rpm, 2.2ml/min, 20mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	571.0	2466.7	100.0
Carotenes enriched alkyl esters	4991.5	2630.7	98.3

2nd Distillation: 150°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Carotenes enriched alkyl esters	4991.5	1293.8	100.0
Carotenes Concentrate	181075.6	1134.7	87.7

Table 9 (Two Stage Distillation – Treatment with Iso-Octane)

1st Distillation: 90^{0}C, 200rpm, 2.2ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	602.0	2340.6	100.0
Carotenes enriched alkyl esters	3720.0	2105.2	89.9

2nd Distillation: 150^{0}C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Treated carotenes enriched alkyl esters	3720.0	964.2	100.0
Carotenes Concentrate	110481.0	851.8	88.3

Note:

MG	: Monoacylglycerol
DG	: Diacylglycerol
TG	: Triacylglycerol
N.D.	: Non-detectable
CPO	: Crude Palm Oil
BDPO	: Bleached and Degummed Palm Oil
Other Minor Components	: Squalene, Sterols, Tocols (tocopherols and tocotrienol)

Table 10 (Re-transesterification of carotenes enriched alkyl esters after first stage distillation-catalyst dissolved in methanol)

	Percentage (%)					
	Esters	**MG**	**DG**	**TG**	**Carotenes**	**Others Minor Components**
CPO Methyl esters	99.413	0.296	0.043	N.D.	0.071	0.177
Carotenes enriched alkyl esters	96.730	0.876	0.509	N.D.	0.632	1.253
Treated carotenes enriched alkyl esters	98.032	0.274	N.D.	N.D.	0.609	1.085

Table 11 (Re-transesterification of carotenes enriched alkyl esters after first stage distillation-catalyst dissolved in treated water)

	Percentage (%)					
	Esters	**MG**	**DG**	**TG**	**Carotenes**	**Others Minor Components**
CPO Methyl esters	99.413	0.296	0.043	N.D.	0.071	0.177
Carotenes enriched alkyl esters	96.730	0.876	0.509	N.D.	0.6320.632	1.253
Treated carotenes enriched alkyl esters	97.740	0.263	0.019	N.D.	0.622	1.357

Table 12 (Two Stage Distillation-with addition of ethyl esters)

2nd Distillation: 150°C, 250rpm, 1mTorr, 3ml/min

	Carotenes		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters + 10% Ethyl Esters	4991.5	998.3	100
Carotenes Concentrate	128120	872.7	87.4

Table 13 (Saponification of carotenes concentrate)

	Percentage (%)						
	FFA	**Esters**	**MG**	**DG**	**TG**	**Carotenes**	**Others**
Carotenes Concentrate	1.45	1.11	34.61	18.26	33.19	2.10	9.28
Unsaponified Sample	24.42	0.00	0.00	13.92	34.95	11.58	15.13

Table 14 (Partition of carotenes)

	Carotenes		
	ppm	mg	%Recovery
Carotenes Concentrate	170982	0.0172	100.0
Hexane Layer	301060	0.0119	69.0
Methanol Layer	58556	0.0036	30.2

Table 15 (Partition of carotenes)

	Carotenes		
	ppm	mg	%Recovery
Carotenes Concentrate	170982	0.0278	100.0
Hexane Layer	243538	0.0235	84.7
Methanol Layer	41274	0.0027	11.6

Table 16

	Phospholipids (%)
Carotenes Concentrate (from Example 1)	1.78
Carotenes Concentrate (from Example 3)	3.83
Carotenes Concentrate (from Example 4)	0.78

Table 18

	Concentration (ppm)	
	Carotenes	**Vitamin E**
Neutralised Palm Oil	512	950
Acidified distilled water washed NPOME	508	908
Normal distilled water washed NPOME	500	921